Kidney International, Vol. 36 (1989), pp. 216–221

Activity of calcium-sensitive phospholipid-dependent protein kinase C following nephron loss

MICHAEL K. HISE and PRAMOD S. MEHTA

The University of Maryland Medical School, Department of Internal Medicine, Division of Nephrology, Baltimore, Maryland, USA

Activity of calcium-sensitive phospholipid-dependent protein kinase C following nephron loss. The localization and activity of the calcium-sensitive phospholipid-dependent protein kinase C (PKC) were examined following the loss of 50% of functioning nephron mass. Four hours following unilateral nephrectomy in rats, soluble (100,000 g supernatant) proteins in the contralateral kidney were increased by 11% compared to sham operated controls; the increase was 33% 144 hours following surgery. The specific activity of PKC did not change in the cytosol at any of the time periods examined and averaged 63.9 ± 8.2 pmol · mg^{-1} · min^{-1} in unilaterally nephrectomized animals four hours following surgery. Four hours following sham surgery total soluble PKC activity averaged 1667.0 ± 278.4 pmol · $kidney^{-1}$ · min^{-1}, whereas activity averaged 3067.7 ± 415.4 pmol · $kidney^{-1}$ · min^{-1} in animals post-nephrectomy ($N = 5$, $P < 0.04$). Similar data was seen 144 hours following surgery. To examine the PKC activity in plasma membranes of proximal tubular cells, brush border membranes were prepared from rat kidney cortex. Twenty-four hours following unilateral nephrectomy, activity averaged 193.8 ± 14.9 pmol · mg^{-1} · min^{-1}, while activity in membranes isolated from sham operated animals averaged 76.6 ± 8.0 pmol · mg^{-1} · min^{-1} ($N = 5$, $P < 0.001$). Similar data was evident 48 hours following surgery. A small increment in activity was seen in the basolateral membrane preparation 24 hours following unilateral nephrectomy but not at 48 hours. These data indicate that cellular PKC activity increases rapidly following reductions in renal mass, and there are selective increments in the brush border membrane of the proximal tubular cell. The localization of PKC to this membrane may have important consequences for adaptations following nephron loss.

The mechanisms underlying cell growth have become increasingly clear with the discovery of multiple growth factors and oncogenes whose products mimic these factors or their receptors [1–4]. Additional insight has been gained with the discovery by Takai et al of the calcium-sensitive phospholipid-dependent protein kinase C (PKC) [5, 6]. Numerous studies of cells in culture indicate that the interaction of some growth promoting factors with their receptors leads to the breakdown of phosphoinositides with the generation of diacylglycerol and inositol triphosphate (IP_3) [7–9]. Diacylglycerol has been shown to lower the requirements of PKC for calcium [10]. Inositol triphosphate stimulates the release of calcium from intracellular stores, presumably the endoplasmic reticulum [11]. PKC has been shown to activate an electroneutral Na^+/H^+ exchanger leading to increased proton extrusion from cells with resultant intracellular alkalinization [12, 13]. These changes acting in concert are thought to play a major role in triggering the biochemical processes associated with growth.

Following reductions in renal mass, the remaining kidney undergoes hypertrophy with an associated increment in functional capacity. The initiating mechanisms of this hypertrophy are unclear although substantial evidence suggests that humoral factors are, in part, responsible [14–17]. The proximal tubule possesses an active Na^+/H^+ exchanger in the luminal membrane which plays an important role in bicarbonate reclamation by the kidney [18]. Prior studies have demonstrated that the luminal membrane also contains significant PKC activity [19]. Since PKC might play a role in the growth response of the kidney, the purpose of this study is to examine the activity and localization of the calcium-sensitive phospholipid-dependent protein kinase C in renal tissue following a reduction in nephron mass.

Methods

Animal and tissue preparation

Male Sprague-Dawley rats, weighing 145 to 185 grams, were used for all studies. Animals underwent either a right sham or right unilateral nephrectomy via a flank incision under ether anesthesia. The wound was closed in two layers and the animals were allowed free access to food and water. After the indicated time intervals, animals were sacrificed and the kidneys homogenized in 7 ml of buffer that contained 250 mM sucrose, 20 mM Tris-HCl (pH 7.5), 0.2 mM EGTA and 0.2 mM EDTA. Twenty strokes of a motor-driven Teflon pestle and a glass tissue grinder tube were used to homogenize the tissue. In studies of cortex, cortical tissue was dissected on a glass plate in an ice bath and the tissue was homogenized in 7 ml of buffer. Homogenates were centrifuged at 100,000 g at 2°C for 60 minutes using a Ti-40 rotor and a Beckman L8-55 ultracentrifuge (Beckman Inst., Fullerton, California, USA). The supernatant fraction was decanted from the firm pellet, and stored briefly on ice. In studies of whole kidney, 500 μl were measured by positive displacement and weighed along with the entire supernatant fraction (Mettler PE 360, Mettler Instruments, Greifensee, Switzerland). The volume of the supernatant was calculated.

Brush border membranes were prepared from rat kidney cortex by standard methods [20, 21]. Basolateral membranes from cortical tissue were prepared by the method of Saktor et al

Received for publication March 3, 1988
and in revised form March 6, 1989
Accepted for publication March 18, 1989

[22]. In studies in which membranes were solubilized, 5 mg of protein were resuspended in 1.0 ml of buffer which contained 20 mM TRIS-HCl (pH 7.5), 0.05 mM EGTA, 0.05 mM EDTA and 1% Nonidet P 40 (Sigma Chemical Co., St. Louis, Missouri, USA). The tubes were incubated on ice for 15 minutes and the mixtures used for protein separation.

Protein separation

Calcium-sensitive phospholipid-dependent protein kinase (PKC) was separated by anion exchange chromatography.

DE-52 (Whatman Ltd., Maidstone, UK) anion exchange resin was equilibrated in buffer that contained 20 mM TRIS-HCl (pH 7.5), 0.05 mM EGTA and 0.05 mM EDTA (Buffer A). Cytosol 2.5 mg or membranes 5 mg were applied to the columns (10 × 0.9 cm) and the columns were washed with 6 ml of buffer A, followed by 3 ml of buffer A that contained 20 mM NaCl. PKC was eluted in 3 ml of buffer A that contained 0.15 mM NaCl.

Protein kinase assay

This assay was designed to measure the transfer of the gamma phosphorus of ATP to histones catalyzed by PKC in the aliquots. PKC activity is stimulated by phosphatidylserine and diolein in the presence of calcium. The incubation volume was 250 μl. Assays were carried out in 13 × 100 mm glass tubes. The media contained 25 mM TRIS-HCl, (pH 7.5), 10 mM $MgCl_2$, 0.1 mM $CaCl_2$, 40 μg of histone V-S (Sigma), and 10 μM γ ^{32}P-ATP (70 to 200 cpm/pmol, New England Nuclear, Boston, Massachusetts, USA). Samples were incubated with and without 20 μM 1,2 diolein and 25 μM phosphatidylserine (Sigma). Phosphatidylserine and diolein were added separately to the incubation media in 20 μl aliquots. Prior to addition, lipids were evaporated to dryness under nitrogen and resuspended in H_2O. Lipids were sonicated by three 20 second bursts on ice under nitrogen using a cup sonicator (Braun-Sonic, B. Braun Inst., San Francisco, California, USA). Assays were carried out in a dry bath incubator at 30°C. For studies of cytosol, 30 μl aliquots were incubated for four minutes. Membranes were studied by assaying 25 μl aliquots for 2.5 minutes. PKC activity was a linear function of both aliquot size and time of incubation. Kinase activity was halted by the addition of 5 ml of ice-cold 7% trichloroacetic acid (TCA). A Millipore ultrafiltration manifold (Millipore Inc., Bedford, Massachusetts, USA) loaded with GF/C 2.4 cm glass fiber filters (Whatman Ltd.) was used to trap phosphorylated histones. The precipitate was washed twice with 5 ml of ice-cold 7% TCA. Filters were allowed to dry and were placed in vials along with 5 ml of Biofluor (New England Nuclear) and radioactivity determined using a Packard 3320 liquid scintillation spectrophotometer (Packard Inst., Downers Grove, Illinois, USA). All assays were carried out in triplicate. Activity in the absence of lipids were subtracted from that in the presence of lipids.

Intravesicular volume

Volumes of membrane vesicles were measured using the equilibrium concentration of (^{3}H)-glucose. Membranes were washed and resuspended in buffer containing 250 mM mannitol, 10 mM TRIS-HCl (pH 7.4) at a concentration of 10 to 12 mg/ml. Ten microliters of this mixture was added to 40 μl of buffer in 13 × 100 mm glass tubes that contained (^{3}H)-glucose, 5200 to 6500 cpm/μl (New England Nuclear). The tubes were allowed to sit at room temperature for 90 minutes, afterwhich 4 ml of ice-cold buffer were added. The mixture was vortexed and filtered over 0.65 μm filters (Millipore, Inc.). The filters were washed twice with 4 ml of buffer and allowed to dry. Five ml of Biofluor were added and radioactivity determined. Non-specific filter binding was measured by incubating tubes with (^{3}H)-glucose but no membranes. Non-specific binding was subtracted from total activity. All studies were done in triplicate.

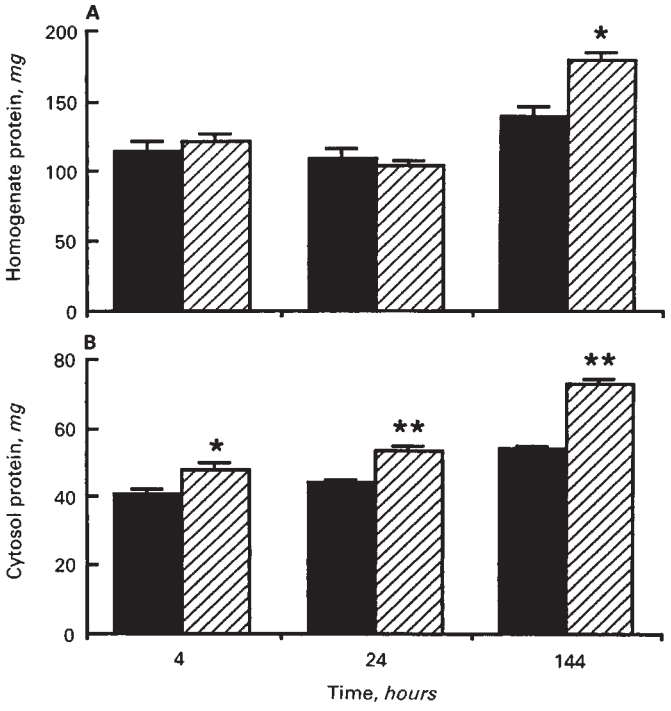

Fig. 1. *Whole kidney homogenate and cytosolic proteins.* Four to five animals were studied in each group. Animals underwent sham (■) or right unilateral (▨) nephrectomy and were sacrificed after the indicated intervals. Cytosol was prepared by centrifugation of whole kidney homogenates at 100,000 g for 60 minutes at 2°C. Abbreviations are: SNx sham nephrectomy, UNx unilateral nephrectomy. Statistical comparisons are between sham and nephrectomized animals at each time period. * $P < 0.03$, ** $P < 0.01$. Data are expressed as means ± SE.

Other analyses

Proteins were determined by the method of Lowry et al [23]. Alkaline phosphatase and acid phosphatase were measured as described in Sigma Technical Bulletin no. 104. Succinate dehydrogenase was measured by the method of King, NADH dehydrogenase by the method of Wallach et al, and Na^+K^+ ATPase as previously described [24–26]. Statistics were performed using the unpaired Student's *t*-test [27].

Results

Animals underwent either sham or unilateral nephrectomy and were sacrificed at 4, 24, or 144 hours following surgery. Animal weights were not different when sham and nephrectomized animals were compared at defined time periods (data not shown). Whole kidney proteins were examined at the indicated time intervals (Fig. 1A). Proteins of nephrectomized animals were elevated when compared to sham operated animals only in

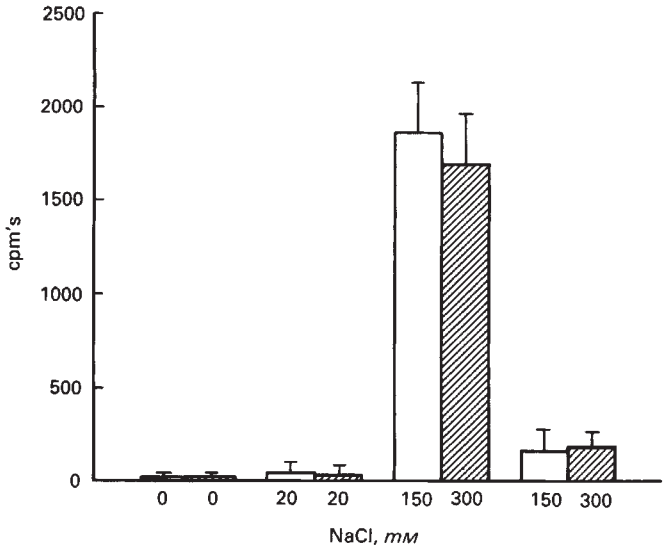

Fig. 2. *Separation of PKC activity.* Brush border membranes which contained 5 mg of protein were solubilized in 1% NP 40 and applied to anion exchange columns. The columns were washed with 6 ml of buffer that contained 20 mM TRIS-HCl (pH 7.5), 0.05 mM EGTA and 0.05 mM EDTA, followed by 3 ml of buffer that contained 20 mM NaCl. PKC activity was eluted in 3 ml of 0.15 M NaCl in buffer. An additional 3 ml were collected. Parallel studies were performed except that columns were eluted with 0.3 M NaCl in buffer. Four separate preparations were studied in each group. PKC activity was measured by a histone assay.

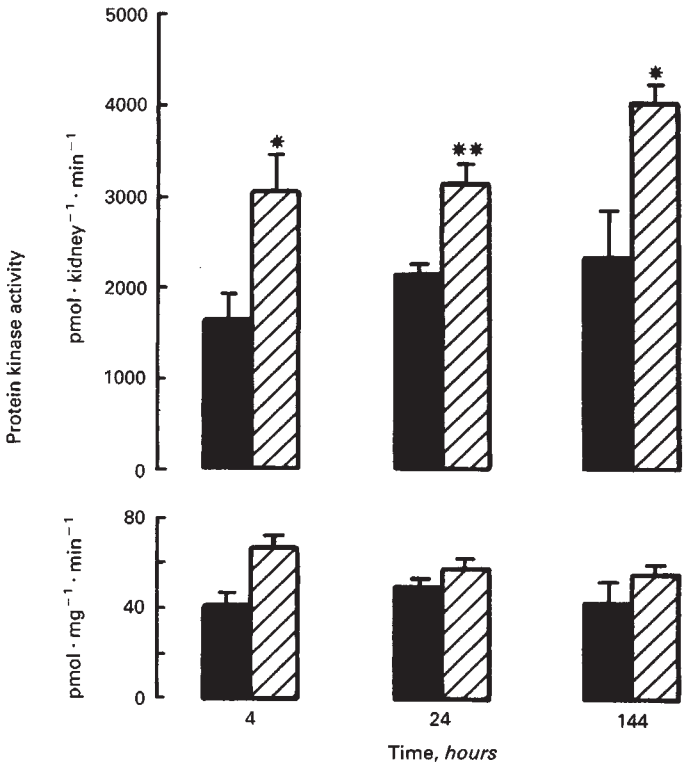

Fig. 3. *Cytosolic PKC activity.* Animals underwent sham (■) or unilateral (▨) nephrectomy and were sacrificed at the indicated intervals. Cytosol was prepared by centrifugation of whole kidney homogenates at 100,000 g for 60 minutes. PKC activity was assayed after the application of 2.5 mg of cytosolic proteins to anion exchange columns. In the lower portion of the figure, activity is expressed per milligram of cytosolic protein. In the upper part of the figure, whole kidney soluble protein activity is depicted. Data are expressed as means ± SE. Four to five animals were studied in each group. * $P < 0.05$, ** $P < 0.01$.

the group 144 hours following surgery. Wet kidney weight averaged 1.410 ± 0.042 g in nephrectomized animals 144 hours following surgery as compared to 1.043 ± 0.076 g in controls ($N = 4$, $P < 0.01$). Because cytosolic proteins may have a different time of appearance when compared to whole kidney proteins, total soluble proteins were examined at 4, 24 and 144 hours following surgery (Fig. 1B). At each time point, proteins from nephrectomized animals were greater than sham operated controls. In separate studies, the membranous protein (100,000 g pellet) content of sham operated animals 144 hours following surgery averaged 109.2 ± 5.2 mg while that of nephrectomized animals averaged 143.7 ± 6.1 mg ($N = 4$, $P < 0.01$). When sham and nephrectomized membranous protein content were examined at 4 or 24 hours following surgery, differences were not apparent (data not shown).

In preliminary studies we attempted to measure PKC activity in cytosol and detergent extracts of membranes; however, despite the use of several detergents including Triton X-100, Nonidet P-40 and octyl-glucoside, lipid stimulated activity was extremely low. Therefore, we used anion exchange columns to partially purify PKC. In order to examine the efficacy of the separation technique for PKC, extracts that contained 5 mg of brush border membrane protein were applied to anion exchange columns. The columns were washed with 6 ml of buffer that contained 20 mM TRIS-HCl (pH 7.5), 0.05 mM EGTA and 0.05 mM EDTA, followed by 3 ml of 20 mM NaCl in buffer. Proteins were eluted in 3 ml of 0.15 M NaCl in buffer. Columns were further eluted with an additional 3 ml. As shown in Figure 2, the first 3 ml of buffer with high salt removed the majority of protein kinase activity. A small portion was removed by an additional 3 ml. Further elution did not result in the removal of more kinase activity (data not shown). Raising the salt concentration to 0.3 M did not result in additional protein kinase activity in either the first or the second elutions. Recovery of PKC activity in the first 3 ml of high salt buffer was greater than 80% of the total. When assayed at 4, 24 or 144 hours following surgery, no alteration in the specific activity of cytosolic PKC could be detected (Fig. 3). However, whole kidney soluble PKC was rapidly elevated. This stimulation was evident at all time periods studied.

To compare brush border and basolateral membrane PKC content following sham or unilateral nephrectomy, these membranes were prepared by standard methods from rat cortical tissue. Brush border membranes were prepared by the magnesium aggregation technique and basolateral membranes by the self-orienting Percoll gradient procedure of Saktor et al [20–22]. To insure that similar membrane populations were being studied, marker enzyme enrichments and balance studies, including measurements of activities in all portions of the preparations, were performed 24 hours following sham or unilateral nephrectomy. As shown in Table 1, alkaline phosphatase was enriched in both the sham and the nephrectomized brush border membrane preparations. A slight enrichment in acid phosphatase was also seen. No significant enrichments of Na^+K^+ ATPase, succinate dehydrogenase or NADH dehydrogenase were ob-

Table 1.

	BLM		BBM	
	SNx	UNx	SNx	UNx
Alkaline phosphatase *μmol/mg/min*				
Homogenate	0.585 ± 0.031	0.592 ± 0.029	0.581 ± 0.081	0.653 ± 0.033
Membrane	0.589 ± 0.056	0.595 ± 0.063	5.535 ± 0.726	6.584 ± 0.809
Enrichment	1.01 ± 0.07	1.02 ± 0.063	9.56 ± 0.31	10.07 ± 0.91
% Recovery	105.4 ± 5.3	95.4 ± 3.1	112.1 ± 9.5	99.9 ± 2.7
Acid phosphatase *nmol/mg/min*				
Homogenate	66.8 ± 9.2	67.0 ± 6.2	77.0 ± 7.9	78.3 ± 9.8
Membrane	71.8 ± 6.9	83.1 ± 9.7	118.5 ± 20.1	118.0 ± 18.9
Enrichment	1.06 ± 0.16	1.27 ± 0.20	1.52 ± 0.17	1.52 ± 0.27
% Recovery	89.3 ± 7.3	87.6 ± 5.7	90.0 ± 4.8	102.9 ± 4.3
NADH dehydrogenase *μmol/mg/min*				
Homogenate	0.517 ± 0.047	0.491 ± 0.034	0.479 ± 0.043	0.471 ± 0.039
Membrane	0.466 ± 0.069	0.484 ± 0.058	0.399 ± 0.052	0.448 ± 0.050
Enrichment	0.90 ± 0.08	0.98 ± 0.06	0.84 ± 0.13	0.96 ± 0.09
% Recovery	111.1 ± 6.0	102.6 ± 5.2	94.1 ± 2.6	101.9 ± 2.3
Succinate dehydrogenase *μmol/mg/min*				
Homogenate	0.175 ± 0.020	0.180 ± 0.016	0.201 ± 0.20	0.225 ± 0.011
Membrane	0.053 ± 0.014	0.068 ± 0.013	0.100 ± 0.008	0.085 ± 0.014
Enrichment	0.31 ± 0.12	0.39 ± 0.08	0.52 ± 0.09	0.38 ± 0.05
% Recovery	99.9 ± 5.7	93.4 ± 2.7	106.4 ± 8.9	100.5 ± 3.1
Na^+ K^+ ATPase *nmol/mg/min*				
Homogenate	4.24 ± 0.49	4.59 ± 0.44	4.00 ± 0.49	5.53 ± 0.65
Membrane	47.63 ± 0.74	43.89 ± 0.26	3.73 ± 0.77	4.55 ± 0.35
Enrichment	11.2 ± 1.5	9.8 ± 1.0	0.94 ± 0.26	0.83 ± 0.11
% Recovery	95.8 ± 10.7	86.5 ± 11.2	87.1 ± 14.9	92.9 ± 7.8

Four to five animals were studied in each group. Data are expressed as means ± SE. Brush border (BBM) or basolateral (BLM) membranes were prepared 24 hours following sham (SNx) or unilateral (UNx) nephrectomy. Differences in enzyme enrichments or percent recoveries were not evident when the two groups were compared for a defined enzyme.

served, suggesting that these preparations were not enriched in basolateral membranes, mitochondria or endoplasmic reticulum. Furthermore, enrichments and recoveries were similar in the sham operated and the nephrectomized animal preparations. When basolateral membranes were studied, there was a nine- to elevenfold enrichment in Na^+K^+ ATPase in these preparations. No clear enrichments of the markers of other membranes were evident, suggesting little contamination of this preparation. Again, enrichments and recoveries were similar when the two groups were compared.

Brush border membrane PKC activity was examined 24 hours following sham or unilateral nephrectomy. As shown in Figure 4, brush border membranes contained significantly more kinase activity 24 hours following unilateral nephrectomy than did brush border membranes prepared from sham-operated control animals. Similar data was evident 48 hours following surgery. More brush border membrane PKC activity was demonstrable 24 hours following nephrectomy than at 48 hours. PKC activity was not affected by exposure to detergent since incubation of cytosol in buffer which contained 1% NP-40 prior to column separation had no discernable effect. PKC activity in control cytosol averaged 63.1 ± 4.8 pmol · mg^{-1} · min^{-1} while activity of cytosol exposed to NP-40 averaged 65.7 ± 8.2 pmol · mg^{-1} · min^{-1} (N = 4). The increase in activity of brush border membranes following unilateral nephrectomy was not related to differences in cytosolic trapping since intravesicular volumes did not change. The intravesicular space averaged 1.8 ± 0.2 μl/mg protein (N = 4) in sham operated animals and 1.4 ± 0.1 μl/mg protein (N = 4) in animals 24 hours post-nephrectomy. Furthermore, cortical cytosolic PKC activity was not

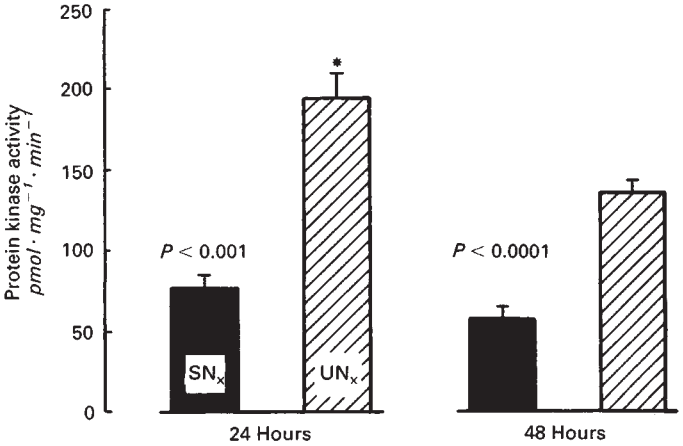

Fig. 4. *Brush border membrane PKC activity.* Kidneys from multiple animals were combined 24 or 48 hours following sham (SNx) or unilateral (UNx) nephrectomy. Brush border membranes were prepared and solubilized in 1% NP-40. PKC activity was assayed after elution from anion exchange columns. Five separate groups were compared at each time interval. * $P < 0.01$, comparison was made between groups 24 and 48 hours following unilateral nephrectomy. Data are expressed as means ± SE.

different 24 hours following surgery. PKC activity in cortical cytosol averaged 70.6 ± 3.0 pmol · mg^{-1} · min^{-1}, while activity in sham operated controls averaged 62.8 ± 11.0 pmol · mg^{-1} · min^{-1} (N = 4). Previous studies have indicated that brush border membrane PKC activity was not different when

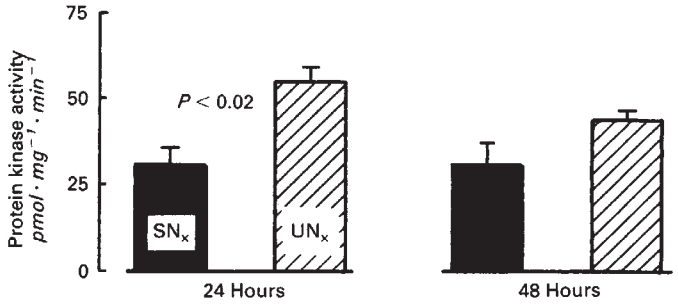

Fig. 5. *Basolateral membrane PKC activity.* Basolateral membranes were prepared from cortical tissue by the method of Sacktor 24 or 48 hours following sham (SNx) or unilateral (UNx) nephrectomy. After solubilization in 1% NP-40, extracts were applied to anion exchange columns and PKC activity eluted in 0.15 M NaCl. Assays were performed immediately. Five separate groups were studied at each interval. Data are expressed as means ± SE.

membranes were prepared in the presence or absence of 2 mM EGTA [19]. Similar data were observed in these studies of renal membranes (data not shown). Illustrated in Figure 5 is the PKC activity of basolateral membranes following sham or unilateral nephrectomy. Increased activity was evident in basolateral membrane preparations 24 hours following unilateral nephrectomy when compared to sham operated controls. However, differences were not apparent 48 hours following surgery.

Discussion

Two major pathways have been implicated in the control of cell growth. Some growth factors act through receptors that have tyrosine kinase activity. Growth is thought to be a result of the phosphorylation of proteins on tyrosine [28]. Other growth factors lead to the hydrolysis of inositol lipids with the production of diacylglycerol and inositol triphosphate [7–9, 29]. As noted earlier, diacylglycerol activates protein kinase C in the presence of calcium and phospholipids, and inositol triphosphate causes the release of calcium from intracellular stores.

Although studies of renal cells in culture have demonstrated that phorbol esters, which activate PKC, can stimulate a rapid increment in sodium phosphate and sodium alanine co-transport, the majority of data suggests that Na^+/H^+ exchange is stimulated by PKC in the plasma membranes of most cells [12, 13, 30, 31]. Activity of canine proximal tubular Na^+/H^+ exchange can also be increased by phorbol esters [32]. Mutants that lack the exchanger cannot grow unless cytoplasmic pH is increased by external means [33]. Increased activity of this exchanger has been noted early following a reduction in renal mass and following hypertrophic stimuli of renal proximal tubular cells in culture [34, 35].

Since PKC may play an important role in the regulation of renal cell mass following nephron loss, the purpose of this study was to examine the activity and the localization of the enzyme after unilateral nephrectomy. The data in Figure 1 indicates that whole kidney proteins did not increase within the first 24 hours following unilateral nephrectomy, but were clearly elevated six days following surgery. Of interest is the observation that cytosolic proteins rise rapidly following nephron loss. This increase in cytosolic proteins is associated with an increment in soluble whole kidney PKC activity (Fig. 3). Brush border membrane PKC activity was studied at 24 and 48 hours following nephrectomy (Fig. 4). At both intervals, PKC was significantly greater in the membranes of animals post-nephrectomy when compared to the membranes of sham-operated control animals. Membrane activity 24 hours following nephrectomy was greater than membrane activity in nephrectomized animals 48 hours following surgery. This increment in activity could not be explained by cytosolic trapping or differences in cortical cytosol activity. Differences also were not related to the membrane preparations under study, since careful enzyme analyses and balance studies (Table 1) demonstrate that similar membrane populations were examined in sham and nephrectomized animals. Basolateral membrane PKC activity was also examined at 24 and 48 hours following nephrectomy (Fig. 5). In these studies, activity was increased in the membranes prepared from animals 24 hours following nephrectomy when compared to sham operated controls but not at 48 hours. These studies must be interpreted with caution, however, since the basolateral membrane preparation is contaminated with brush border membranes (~15%). Because of the very large rise in brush border membrane activity, no clear picture of the activity in the basolateral membrane can be obtained. Two facts are obvious, first the basolateral membrane contains less PKC activity than does the brush border membrane, results which confirm previous studies, and second, any increments of activity are not stimulated to the same magnitude when compared to the brush border membrane [19].

The higher activity of PKC in the brush border membrane when compared to the basolateral membrane, and the increments seen following a reduction in renal mass suggest that in proximal tubular cells, the primary location of PKC's actions are at the luminal membrane. Since most receptors are located in the basolateral membrane of the proximal tubular cell, further studies will be necessary to understand how the activity of this protein is regulated. The presence of increased quantities of PKC in the luminal membrane following nephron loss may have important implications for growth and the regulation of membrane transport by the metabolism of intermediate proteins.

Acknowledgments

The authors would like to thank Miss Donna DeLuca for transcribing the manuscript.

Reprint requests to Dr. Michael K. Hise, The University of Maryland Hospital, Division of Nephrology, 22 South Greene Street, Baltimore, Maryland 21201, USA.

References

1. James R, Bradshaw RA: Polypeptide growth factors. *Ann Rev Biochem* 53:259–292, 1984
2. Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, Ullrich A, Schlessinger J, Waterfield MD: Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. *Nature* 307:521–527, 1984
3. DeLarco JE, Todaro GJ: Growth factors from murine sarcoma virus-transformed cells. *Proc Natl Acad Sci USA* 75:4001–4005, 1978
4. Ozanne B, Fulton RJ, Kaplan PL: Kirsten murine sarcoma virus transformed cell lines and a spontaneously transformed rat cell-line produce transforming factors. *J Cell Physiol* 105:163–180, 1980

5. TAKAI Y, KISHIMOTO A, INOUE M, NISHIZUKA Y: Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. *J Biol Chem* 252:7603–7609, 1977
6. TAKAI Y, KISHIMOTO A, IWASA Y, KAWAHARA Y, MORI T, NISHIZUKA Y: Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *J Biol Chem* 254: 3692–3695, 1979
7. HABENICHT AJR, GLOMSET JA, KING WC, NIST C, MITCHELL CD, ROSS R: Early changes in phosphatidylinositol and arachidonic acid metabolism in quiescent Swiss 3T3 cells stimulated to divide by platelet-derived growth factor. *J Biol Chem* 256:12329–12335, 1981
8. BERRIDGE MJ, HESLOP JP, IRVINE RF, BROWN KD: Inositol trisphosphate formation and calcium mobilization in Swiss 3T3 cells in response to platelet-derived growth factor. *Biochem J* 222:195–201, 1984
9. TAKUWA N, TAKUWA Y, BOLLAG WE, RASMUSSEN H: The effects of Bombesin on polyphosphoinositide and calcium metabolism in Swiss 3T3 cells. *J Biol Chem* 262:182–188, 1987
10. KISHIMOTO A, TAKAI Y, MORI T, KIKKAWA U, NISHIZUKA Y: Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. *J Biol Chem* 255:2273–2276, 1980
11. BERRIDGE MJ, IRVINE RF: Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* 312:315–321, 1984
12. MOOLENAAR WH, TERTOOLEN LGJ, DE LAAT SW: Phorbol ester and dicylglycerol mimic growth factors in raising cytoplasmic pH. *Nature* 312:371–374, 1984
13. SWANN K, WHITAKER M: Stimulation of the Na/H exchanger of sea urchin eggs by phorbol ester. *Nature* 314:274–277, 1985
14. LOWENSTEIN LM, STERN A: Serum factor in renal compensatory hyperplasia. *Science* 142:1479–1480, 1963
15. OBERTOP H, MALT RA: Lost mass and excretion as stimuli to parabiotic compensatory renal hypertrophy. *Am J Physiol* 232: F405–F408, 1977
16. YAMAMOTO N, KANETAKE H, YAMADA J: In-vitro evidence from tissue cultures to prove the existence of rabbit and human renotropic growth factor. *Kidney Int* 23:624–631, 1983
17. HARRIS RH, HISE MK, BEST CF: Renotrophic factors in urine. *Kidney Int* 23:616–623, 1983
18. SEIFTER JL, ARONSON PS: Properties and physiological roles of the plasma membrane sodium-hydrogen exchanger. *J Clin Invest* 78: 859–864, 1986
19. BARRETT PQ, ZAWALICH K, RASMUSSEN H: Protein kinase C activity in renal microvillus membranes. *Biochem Biophys Res Comm* 128:494–505, 1985
20. ARONSON PS: Energy-dependence of phlorizin binding to isolated renal microvillus membranes. *J Membr Biol* 42:81–98, 1978
21. BLOMSTEDT JW, ARONSON PS: pH gradient stimulated transport of urate and p-aminohippurate in dog renal microvillus membrane vesicles. *J Clin Invest* 65:931–934, 1980
22. SAKTOR B, ROSENBLOOM IL, LIANG CT, CHENG L: Sodium gradient and sodium plus potassium gradient-dependent L-glutamate uptake in renal basolateral membrane vesicles. *J Membr Biol* 60:63–71, 1981
23. LOWRY OH, ROSENBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265–275, 1951
24. KING TE: Preparation of succinate-cytochrome a reductase. *Meth Enzymol* 10:216–225, 1967
25. WALLACH DFH, KAMAT VB: Preparation of plasma membrane fragments from mouse ascites tumor cells. *Meth Enzymol* 8:164–172, 1966
26. KAHN AM, BRANHAM S, WEINMAN EJ: Mechanism of urate and p-aminohippurate transport in rat renal microvillus membrane vesicles. *Am J Physiol* 245 (Renal Fluid Electrol Physiol 14): F151–F158, 1983
27. SNEDECOR GW, COCHRAN WG: *Statistical Methods* (6th ed). Ames, Iowa State University Press, 1967, p. 59
28. CARPENTER G: Receptors for epidermal growth factor and other polypeptide mitogens. *Ann Rev Biochem* 56:881–914, 1987
29. ROZENGURT E, RODRIGUEZ-PENA A, SINNETT-SMITH J: Signalling mitogenesis in 3T3 cells: Role of Ca^{2+}-sensitive, phospholipid-dependent protein kinase, in *Growth Factors in Biology and Medicine*, edited by EVERED D, NUGENT J, WHELAN J, London, Pitman, 1985, p. 66
30. KINOSHITA Y, FUKASE M, YAMATANI T, HISHIKAWA R, FUJITA T: Phorbol esters stimulate phosphate accumulation synergistically with A23187 in cultured renal tubular cells. *Biochem Biophys Res Comm* 136:177–182, 1986
31. MOHRMANN I, MOHRMANN M, BIBER J, MURER H: Stimulation of Na^+/phosphate cotransport in LLC-PK_1 cells by 12-0-tetradecanoylphorbol 13-acetate (TPA). *Biochem Biophys Acta* 860:35–43, 1986
32. MELLAS J, HAMMERMAN, MR: Phorbol ester-induced alkanlinization of canine renal proximal tubular cells. *Am J Physiol* 250: F451–F459, 1986
33. POUYSSEGUR J, SARDET C, FRANCHI A, L'ALLEMAIN G, PARIS S: A specific mutation abolishing Na^+/H^+ antiport activity in hamster fibroblasts precludes growth at neutral and acidic pH. *Proc Natl Acad Sci USA* 81:4833–4837, 1984
34. HARRIS RC, SEIFTER JL, BRENNER BM: Adaptation of Na^+-H^+ exchange in renal microvillus membrane vesicles. Role of dietary protein and uninephrectomy. *J Clin Invest* 74:1979–1987, 1984
35. FINE LG, BADIE-DEZFOOLY B, LOWE AG, HAMZEH A, WELLS J, SALEHMOGHADDAM S: Stimulation of Na^+/H^+ antiport is an early event in hypertrophy of renal proximal tubular cells. *Proc Natl Acad Sci USA* 82:1736–1740, 1985